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A compound heterozygous mutation in the *CYP17* (17α-hydroxylase/17,20-lyase) gene in a Chinese subject with congenital adrenal hyperplasia

Gin-Sing Won[a,b], Chih-Yang Chiu[c,d], Yi-Chu Tso[c,d], Shwu-Fen Jenq[c,d], Pi-Sung Cheng[a,b], Tjin-Shing Jap[a,b,c,d,*]

[a]*Division of Endocrinology and Metabolism, Veterans General Hospital-Taipei, Taiwan, ROC*
[b]*School of Medical Technology and Engineering, National Yang-Ming University, Taiwan, ROC*
[c]*Section of Biochemistry, Department of Pathology and Laboratory Medicine, Veterans General Hospital-Taipei, Taiwan, ROC*
[d]*School of Medical Technology and Engineering, National Yang-Ming University, Taiwan, ROC*

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Abstract

Mutations in the *CYP17* gene impair steroid biosynthesis in the adrenals and gonads, resulting in 17α-hydroxylase/17,20-lyase (P450c17) deficiency, leading to amenorrhea, sexual infantilism, hypokalemia, and hypertension. To date, more than 50 mutations in the *CYP17* gene associated with congenital adrenal hyperplasia have been described. In this study, we analyzed a 36-year-old phenotypic female, genotypic male, with P450c17 deficiency to compare with an additional group of 50 Chinese subjects without P450c17 deficiency in Taiwan. DNA sequence analysis of the *CYP17* gene was performed. The result showed that the proband had a compound heterozygous mutations in exon 6 (CGC→TGC) that resulted in the substitution of arginine by cysteine at codon 362, and in exon 7 (CCG→CGG) that resulted in the substitution of proline by arginine at codon 409. In conclusion, we have identified a compound heterozygous mutation in the *CYP17* gene in one patient with congenital adrenal hyperplasia in Taiwan.

1. Introduction

Congenital adrenal hyperplasia (CAH) refers to a group of autosomal recessive disorders characterized by defects in the synthesis of cortisol. Depending on the enzymes involved, the synthesis of other steroids such as mineralocorticoids and sex steroids may be affected [1]. Mutations in the *CYP17* gene impair steroid biosynthesis in the adrenals and gonads, resulting in 17α-hydroxylase/17,20-lyase (P450c17) deficiency, leading to amenorrhea, sexual infantilism, hypokalemia, and hypertension [1,2].

The microsomal, 56-kDa steroidogenic enzyme P450c17 is highly expressed in both zona fasciculata and zona reticularis of the adrenal cortex as well as the Leydig and theca cells of the gonads [3]. It plays a key role in the biosynthesis of steroid hormones by catalyzing 2 unique reactions: 17α-hydroxylase and 17,20-lyase [3]. Through P450c17 hydroxylation, pregnenolone is converted into 17α-hydroxypregnolone, and progesterone (PGTR) into 17α-hydroxyprogesterone. These 2 products may be further cleaved by 17,20-lyase to generate dehydroepiandrosterone and androstenedione, respectively. Both the hydroxylation and cleavage functions are catalyzed sequentially at the common active site of P450c17 and proceed through transfer of 2 electrons from the reduced form of nicotinamide adenine dinucleotide phosphate via its redox partner, cytochrome P-450 reductase [3,4].

The majority of P450c17 kindreds have their genetic abnormality linked to chromosome 10q24.3, the locus for the *CYP17* gene [5,6]. In the absence of P450c17 activity (as in the zona glomerulosa), aldosterone can be formed and there would be no synthesis of cortisol and sex steroids [1,2]. To date, more than 50 different mutations in the *CYP17* gene leading to inactivation of the hydroxylase and/or lyase activities have been identified (www.hgmd.cf.ac.uk/ac/gene.php?gene=CYP17A1). These mutations occur

* Corresponding author. Section of Biochemistry, Department of Pathology and Laboratory Medicine, Veterans General Hospital-Taipei 112, Taiwan. Tel.: +886 2 28757158; fax: +886 2 28717680.
E-mail address: tsjap@vghtpe.gov.tw (T.-S. Jap).

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Table 1
Oligonucleotide primers used for PCR amplification, PCR product sizes, and annealing temperatures

Exon	Forward primer	Reverse primer	Product size (bp)	Annealing temperature (°C)
1	TTGCCACAGCTCTTCTACTC	TCTGAAGACCTGAACCAATC	419	55
2	TGGGTGTGAGATTCCTACAG	TCCTAACCCCTTACCCCTG	269	55
3	TGGTACAGAGAGGGGGTAAG	GGGACAATGTCAGGGTCTAC	354	55
4	AGCTAAGATCCGCCTCCAG	TCCACCCTGCTCTTGTGATT	236	55
5	GGCAGGAGTGTCACAGATG	TGGGGTCTAGGATCAATGAG	311	55
6	ACACACTAGTCACCTCCAAC	TGAATGCATCATGGGGCTAG	250	55
7	ACTTTTCCTCTTCCACTCTG	TTGCAGAGGTGAAGGGGTA	215	55
8	TCAACCAGGGCAGAACCATG	TGTGTTGTGGGGCCACATAG	404	55

Adapted from Manno et al (1993).

throughout the coding and noncoding regions of the gene, including missense, nonsense mutations, deletion, insertion, and splice site mutations. All are predicted to impair the hydroxylation and cleavage activities [1,2]. The degree of inactivation is dependent on the type and localization of the mutation in the gene.

Reports of *CYP17* gene mutations are uncommon among patients with CAH in Asian populations studied, including Japanese, Korean, Chinese, and Thai patients [7-13]. We report here a Han Chinese in Taiwan with 17α-hydroxylase/17,20-lyase deficiency carrying a compound heterozygous mutation in the *CYP17* gene.

2. Research design and methods

2.1. Subjects

Genomic DNA from 1 patient with 17α-hydroxylase/17,20-lyase deficiency and from her parents was analyzed. An additional group of 50 Chinese subjects without P450c17 deficiency were recruited as controls to test whether it is a common polymorphism. The study protocol was approved by the institutional review board of the hospital, and informed consent was obtained from each patient and normal subjects.

2.2. Polymerase chain reaction amplification

The 8 coding exons of the *CYP17* gene were amplified by polymerase chain reaction (PCR) using primers and conditions as previously described (Table 1) [14].

2.3. Sequence analysis of CYP17 gene

The coding exons plus the exon-intron junctions of *CYP17* from the affected patient was amplified by PCR and the purified amplicons were subjected to automated DNA sequencing (ABI 377-36 Autosequencer, Perkin Elmer, Foster City, CA) according to the manufacturer's recommendations. Exons 6 and 7 of *CYP17* were also sequenced in the parents.

2.4. Mutation confirmation

We used restriction analysis to confirm the presence of mutations in the *CYP17* gene. Restriction endonucleases were selected on the basis of whether a mutation creates or destroys a restriction endonuclease site. If a mutation neither creates nor destroys a restriction endonuclease site, we would use modified PCR primers to introduce base substitutions adjacent to a codon of interest and thereby create an artificial restriction site on only one allelic form (wild or mutant). We amplified exon 6 of *CYP17* gene with a modified primer that created a recognition site only if the codon contained the wild-type sequence (Fig. 1A). Digestion of PCR products was analyzed by electrophoresis through a 3.5% agarose gel with fragments visualized by ethidium bromide.

2.5. Case report

A 36-year-old phenotypic female, genotypic male, visited the hospital because of general weakness. Her parents have no apparent clinical abnormalities and are currently in good health. She sought medical attention in 1986 at the age of 15 because of primary amenorrhea. She underwent a laparascopic evaluation for absence of uterus and presence of undescended testes and underwent orchiectomy 4 years later in 1990. The chromosome study revealed an XY karyotype. At the age of 24 in 1994, she experienced an episode of adrenal crisis with clinical presentation of high fever, fainting spell, and inability to walk. She was confirmed as a case of CAH with adrenal insufficiency. She had been taking 7.5 mg prednisolone daily ever since.

Recently, she again underwent a comprehensive evaluation of CAH in our institution, which disclosed the following values (normal values in parentheses): plasma sodium, 140 mmol/L (135-147); potassium, 4.4 mmol/L (3.4-4.7); PGTR, 5.4 nmol/L (follicular phase, <3.8); 17α-hydroxyprogesterone, less than 0.3 nmol/L (follicular phase, 0.3-3.1); 11-deoxycorticosterone, 3.77 nmol/L (0.23-0.81); corticosterone, 709 nmol/L (1-23); aldosterone, 0.38 nmol/L (0.03-0.44); renin, 0.28 pmol/L (0.07-0.38); dehydroepiandrosterone, less than 140 nmol/L (female, 108-1070); estradiol, 17 pmol/L (follicular phase, 91-440); FSH, 31.82 IU/L (follicular phase, 1.8-10.5); LH, 31.27 IU/L (follicular phase, 0.5-5); testosterone, less than 0.48 nmol/L (female, 0.31-2.98); and cortisol, less than 2.8 nmol/L (119-618). The serum cortisol failed to response to corticotropin infusion (data not shown). On physical examination she was 169 cm in height, 76 kg in weight, and had normotension. Lack of sexual maturation including absence of axillary and pubic

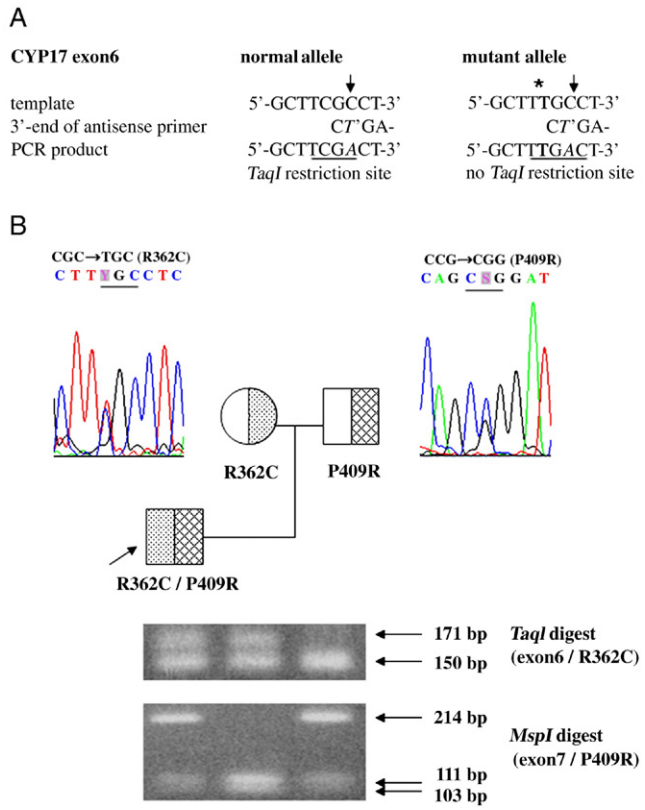

Fig. 1. A, Strategy for insertion of diagnostic *Taq*I restriction site to detect the *CYP17* R362C mutation. The arrows indicate the nucleotide mismatch. *Indicates the position of the *CYP17* mutation. B, Pedigree of the family with *CYP17* gene mutation. Affected patients are indicated by solid symbols. The presently asymptomatic individuals carrying a mutation are indicated by shaded symbols. The arrow indicates the proband. The size of the PCR product of exon 7 is 214 base pairs (bp). The mutation destroys the *Msp*1 endonuclease site. After *Msp*1 treatment, 2 restriction products of 103 and 111 bp in the wild type and an undigested 214-bp fragment in P409R mutant were produced. The size of the PCR product of exon 6 is 171 bp. After *Taq*I treatment, 2 restriction products of 150 and 21 bp in the wild type and an undigested 171-bp fragment in R362C mutant were produced. The 21-bp fragment has run off the gel.

hair, breasts, and clitoris was observed (Tanner stage 1). A previous operation scar over the lower mid abdomen was noted. Computed tomography scan of the abdomen showed normal size and appearance of both adrenal glands, non-visualization of the uterus, and a blind vagina pouch. The biochemical and clinical evaluation was mostly consistent with partial combined 17α-hydroxylase/17,20-lyase deficiency, and thus we analyzed her *CYP17* gene.

3. Results

The result showed that the proband had a compound heterozygous mutation in exon 6 (CGC→TGC) that resulted in the substitution of arginine by cysteine at codon 362, and in exon 7 (CCG→CGG) that resulted in the substitution of proline by arginine at codon 409. Both restriction analysis and direct sequencing study confirmed that the R362C and P409R mutations were inherited from her mother and father, respectively (Fig. 1B). Neither R362C nor P409R mutation was found in 50 normal subjects. The R362C mutation neither creates nor destroys any endonuclease restriction site, so we used modified PCR primers to introduce base substitutions adjacent to a codon of interest and thereby create an artificial restriction site for *Taq*I only on allelic wild form (Fig. 1A). P409R mutation destroys the *Msp*1 restriction site.

4. Discussion

Although clinically diagnosed mutations of *CYP17* gene may exhibit impairment of both hydroxylation and cleavage activities or of isolated cleavage activity, some individuals may also present with normal 17α-hydroxylase activity throughout childhood and adolescence that then decreases in adulthood [15]. Similarly, the proband presented with normal 17α-hydroxylase activity throughout childhood and adolescence but then decreased abruptly at the age of 24.

Because there were normal aldosterone level, elevated corticosterone level, no hypokalemia, no hypertension during reevaluation, and the history of adult-onset adrenal crisis, the proband was a case of partial combined 17α-hydroxylase/17,20-lyase deficiency. On the other hand, some patients, particularly those of Japanese origin [2,8], may present as hyperaldosteronism. In other expression studies, it was determined in all complete deficiency cases that both activities were diminished to the same extent and confirmed that the *CYP17* enzyme must retain about one fourth of its catalytic capability to prevent the onset of mineralocorticoid-dependent hypertension [16].

The 2 missense mutations, R362C and P409R, have been separately reported before, the R362C in a Brazilian subject and the P409R mutation in a Chinese subject. The 2 mutations have been previously analyzed by heterologous expression, and the mutation-related biological consequences have been extensively discussed [10,17,18].

To date, more than 20 missense mutations have been identified in *CYP17*. Several of them are known to have mechanisms that affect the enzyme activity. The severity of hypertension, hypokalemia, 17-deoxysteroid excess, and sex steroid deficiency varied, even among patients with completely inactive P450c17 protein(s) [18].

In conclusion, the compound heterozygous mutations of R362C and P409R have been reported in 2 separate patients, but here is first reported in the same patient.

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